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Product Information

ExoBrite™ EV Membrane Staining Kits

Catalog Number: See Table 1

Kit Contents

Component	**Full Size** 500 labelings	**Trial Size** 100 labelings
ExoBrite™ EV Membrane Stain	Component A 5 vials	Component A 1 vial
ExoBrite™ Reconstitution Solution	99858 1 mL	99858 1 mL

Storage and Handling

Store at -20°C upon arrival and protect from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Reconstitution

To prepare 500X ExoBrite™ stain solution, dissolve one vial of Component A in 100 uL of ExoBrite™ Reconstitution Solution. Pipet gently up and down to mix. The 500X stain solution can be stored protected from light for at least 2 months at either 4°C or -20°C.

Note: ExoBrite™ Reconstitution Solution contains 0.05% sodium azide.

Spectral Properties

See Table 1.

Product Description

Extracellular vesicles (EVs), including exosomes, are lipid-bound vesicles that are released from cells. EVs display specific surface proteins and can carry nucleic acids and other cargo, allowing them to transfer biological information between cells in different parts of the body. Therefore exosomes are increasingly studied for their potential use in drug delivery and medical diagnostic applications. Biotium developed ExoBrite™ EV Membrane Stains for fluorescent labeling and detection of EVs and exosomes by flow cytometry. Other potential applications include fluorescence microscopy and other fluorescence detection platforms.

ExoBrite™ stains were designed to overcome some of the challenges of exosome detection, particularly in flow cytometry. While some dyes may form aggregates of a similar size as exosomes or EVs, thus confounding analysis, ExoBrite™ stains show little to no background, allowing exosomes to be accurately identified. ExoBrite™ stains bind to molecules in the exosome membrane for bright, specific staining. Unlike most other membrane stains, ExoBrite™ stains do not bind non-specifically to polystyrene beads, meaning that they can be used to stain bead-bound exosomes.

Exosomes are often labeled with fluorescent antibodies targeting one or more of the tetraspanin proteins CD9, CD63, and CD81. ExoBrite™ staining can be combined with antibody staining, for multi-parameter analysis (see Staining Protocol). Biotium offers CD9 and CD81 monoclonal antibodies validated in exosome staining, available purified or conjugated to CF® Dyes (see Related Products).

Considerations for Detecting Exosomes by Flow Cytometry

- Exosomes are extremely small vesicles (~30-150 nm in diameter), a size which is near or below the size detection limit of some flow cytometers. We recommend determining the size detection limit of your instrument by running sizing beads (for example, ranging from 0.02-2 um) in SSC before attempting to detect purified exosomes. We also recommend running sizing beads before each exosome detection experiment, and using them to set the SSC threshold. Exosomes that are bound to affinity beads are large enough to detect on any instrument.
- Consider using a 405 nm laser for the SSC instead of a 488 nm laser, for improved sensitivity for small particles.
- Use a low flow rate to keep the event rate and abort rate low. This will result in reduced instrument noise. Dilute the stained samples in filtered PBS if necessary.
- For best results, buffers used for suspending and staining exosomes should be filtered through a 0.2 um filter to remove particulates.

Considerations for Staining With ExoBrite™ EV Membrane Stains

The following are general considerations for using ExoBrite™ to stain exosomes or EVs. See Experimental Protocols for step-by-step instructions for use.

- ExoBrite™ EV Membrane Stains have been validated in flow cytometry on both the CytoFLEX LX from Beckman Coulter and the LSRII from BD. Results on other instruments may vary based on the instruments size detection limit and other parameters.
- Individual exosomes and EVs are too small to be imaged by conventional fluorescence or confocal microscopy, but clusters of EVs taken up by cells may be visualized. ExoBrite™ EV Membrane Stains have not been validated for labeling exosomes for cellular uptake. It may be necessary to remove free stain (by ultrafiltration, for example) before attempting to apply ExoBrite™-labeled exosomes to cells.
- Exosomes can be imaged by super-resolution microscopy. The ExoBrite™ 410/450 fluorophore is compatible with SIM and STED. The ExoBrite™ 490/515 fluorophore is compatible with STED, STORM, and TIRF. The ExoBrite™ 560/585 fluorophore is compatible with SIM, STED, and STORM.
- ExoBrite™ EV Membrane Stains have been validated for staining exosomes isolated using several different methods, including PEG precipitation, size excusion chromatography, and affinity bead isolation. Staining results may vary depending on the exosome isolation method used.
- While we have found that staining with 1X ExoBrite™ EV Membrane Stain gives a bright signal and low background under our typical staining conditions, we have also seen excellent results at concentrations between 1X and 100X. The dye concentration may require optimization for different samples and detection systems.
- ExoBrite™ EV Membrane Stains can be used for co-staining with fluorescently labeled primary antibodies. Co-staining can be performed concurrently or sequentially (see “Antibody Co-Staining of Purified Exosomes” under Experimental Protocols).

Table 1. ExoBrite™ EV Membrane Staining Kits

Cat. No.	Size	Product Name	Dye Ex/Em	Laser Line(s) (nm)	Detection Channel
30111	500 labeling reactions	ExoBrite™ 410/450 EV Membrane Staining Kit	416/452 nm	405	Pacific Blue™
30111-T	100 labeling reactions				
30112	500 labeling reactions	ExoBrite™ 490/515 EV Membrane Staining Kit	490/516 nm	488	FITC
30112-T	100 labeling reactions				
30113	500 labeling reactions	ExoBrite™ 560/585 EV Membrane Staining Kit	562/584 nm	488, 532, or 561	PE
30113-T	100 labeling reactions				
30114	500 labeling reactions	ExoBrite™ 640/660 EV Membrane Staining Kit	642/663 nm	633-640	APC
30114-T	100 labeling reactions				

Experimental Protocols

Note: Before beginning, please read "Considerations for Staining EVs with ExoBrite™ EV Membrane Stains" on previous page.

Staining Purified Exosomes

This protocol was developed for staining purified exosomes with ExoBrite™ EV Membrane Stains for detection by flow cytometry.

1. Isolate or purify EVs or exosomes using the procedure of your choice.
2. Aliquot 100 uL of exosomes into FACS tubes or microcentrifuge tubes.
3. Prepare 1X ExoBrite™ staining solution by diluting the 500X stock solution 1:500 in 1X PBS (e.g., add 2 uL ExoBrite™ stain to 1 mL PBS).

   Note: The concentration of ExoBrite™ stain can be optimized by the user; we find that concentrations ranging from 1X to 100X give good signal.
4. In addition to the ExoBrite™-stained exosome samples, it is helpful to include the following controls (the buffer should be an appropriate negative control for the exosomes, such as a mock purification or the buffer used to suspend the exosomes):
   a. Buffer alone (no exosomes, no stain)
   b. Buffer plus ExoBrite™
   c. Exosomes alone (no stain)
5. Add 900 uL of 1X ExoBrite™ staining solution to each tube containing 100 uL sample. Remember to also add the staining solution to the "buffer plus ExoBrite™" control.
6. Incubate at room temperature for 30 minutes, protected from light.
7. Run the samples on a flow cytometer. For tips for flow cytometry detection of purified exosomes read "Considerations for Detecting Exosomes by Flow Cytometry" on page 1.

Antibody Co-staining of Purified Exosomes

This protocol was developed for staining purified exosomes with both ExoBrite™ EV Membrane Stains and fluorescent antibodies, and detecting them by flow cytometry.

Note: Use labeled primary antibodies at the manufacturer's recommended concentration, or try staining in the range of 0.1-5 ug/mL. Either co-incubation or sequential incubations can be performed as described below.

1. Follow steps 1-3 in the "Staining Purified Exosomes" protocol above. In addition to the antibody and ExoBrite™ co-stained exosome samples, it is helpful to include the following controls (if using multiple antibodies, include "buffer plus antibody" and single-stain controls for each antibody).

   Buffer controls
   a. Buffer alone (no exosomes, no stain)
   b. Buffer plus ExoBrite™
   c. Buffer plus antibody

   Exosome controls
   a. Unstained exosomes
   b. Single-stain ExoBrite™
   c. Single-stain antibody
2. Choose whether to co-stain by co-incubation (proceed to step 3) or sequential incubation (proceed to step 4).
3. Co-incubation of antibodies and ExoBrite™:

   a. Add 900 uL of 1X ExoBrite™ staining solution to each tube containing 100 uL of exosomes. Remember to also add the staining solution to the "buffer plus ExoBrite™" control and the ExoBrite™ single-stain control tubes.

   b. Add fluorescent antibody conjugate to the samples at the desired concentration. For example, to the 1 mL staining reaction, add 1 ug antibody for 1 ug/mL. Remember to also add the antibody to the "buffer plus antibody" control and the antibody single-stain control tubes.

   c. Continue to steps 6-7 in the "Staining Purified Exosomes" protocol.
4. Sequential incubation of antibodies and ExoBrite™:

   a. Add fluorescent antibody conjugate to the samples at the desired concentration. For example, to the 100 uL exosome sample, add 0.1 ug antibody for 1 ug/mL. Remember to also add the antibody to the "buffer plus antibody" control and the antibody single-stain control tubes.

   b. Incubate at room temperature for 30 minutes, protected from light.

   c. Add 900 uL of 1X ExoBrite™ staining solution to each sample tube. Remember to also add the staining solution to the "buffer plus ExoBrite™" control and the ExoBrite™ single-stain control tubes.

   d. Continue to steps 6-7 in the "Staining Purified Exosomes" protocol.

Staining Bead-Bound Exosomes

This protocol was developed for exosomes bound to magnetic antibody capture beads, stained with ExoBrite™ EV Membrane Stains, and detected by flow cytometry.

1. Prepare exosomes bound to the magnetic capture beads of your choice, according to the manufacturer's recommended procedure.
2. Prepare the following control tubes:
   a. Beads alone (no exosomes or stain)
   b. Beads plus ExoBrite™ (no exosomes)
3. Prepare 10X ExoBrite™ staining solution by diluting the 500X stock solution 1:50 in 1X PBS (e.g., add 2 uL ExoBrite™ stain to 100 uL PBS).
4. Place the tubes with beads on a magnet for 1 minute, remove and discard the supernatant.
5. Remove the tubes with beads from the magnet and suspend in 100 uL of 10X ExoBrite™ staining solution. Remember to also add the staining solution to the "beads plus ExoBrite™" control.
6. Incubate at room temperature for 30 minutes, protected from light.
7. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
8. Remove the beads from the magnet, add 100 uL of sterile-filtered PBS and gently pipet up and down to resuspend.
9. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
10. Remove the tubes from the magnet, add 500 uL of sterile-filtered PBS and gently pipet up and down to resuspend.
11. Run the samples on a flow cytometer.

Related Products

Catalog number	Product
BNUB1619	CD9 Monoclonal Mouse Antibody (clone CD9/1619) CF® Dye and Other Conjugates
BNUB2343	CD9 Monoclonal Mouse Antibody (clone CD9/2343) CF® Dye and Other Conjugates
BNUB0391	CD81 Monoclonal Mouse Antibody (clone 1.3.3.22) CF® Dye and Other Conjugates
BNUB3442	CD81 Recombinant Monoclonal Mouse Antibody (clone rC81/3442) CF® Dye and Other Conjugates

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

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